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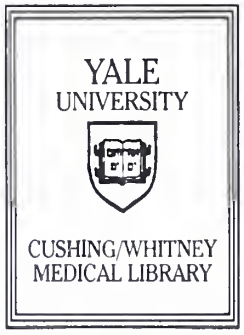
URINARY CATECHOLAMINES AND CORTISOL IN ATTENTION DEFICIT/HYPERACTIVITY DISORDER

Marcia Dover

1998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Urinary Catecholamines and Cortisol in Attention Deficit/Hyperactivity Disorder

A Thesis submitted to the

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Abstract

URINARY CATECHOLAMINES AND CORTISOL IN ATTENTION DEFICIT/ HYPERACTIVITY DISORDER. Marcia Dover, John Holahan, George Anderson, Bennett Shaywitz, Karen Marchione, Sally Shaywitz. Department of Pediatrics, Yale University, School of Medicine, New Haven, CT.

Our aim was to examine urinary catecholamines and cortisol excretion in a large sample of children with ADHD of varying subtypes, looking for differences between the sub-types and with respect to normal controls. We also sought to establish the effect of comorbid Learning Disorder (LD) or Oppositional Defiant Disorder/Conduct Disorder in ADHD, and to replicate previous findings regarding urinary epinephrine excretion in ADHD. Boys (n = 373) between 7.5 and 13.5 years of age were recruited. Tests of attention, impulsivity and visio-spatial tasks were administered during the course of a three-hour urine collection period. Urinary excretion rates for norepinephrine, epinephrine, dopamine and cortisol were determined. A two-way ANOVA was used to examine urinary epinephrine excretion in children with ADHD compared with normal children. A MANOVA was used to examine possible relationships between the catecholamines and ADHD, and ANOVA was used to examine possible relationships between cortisol and ADHD. Urinary epinephrine levels were significantly lower in children with ADHD compared with normal children ($F_{1,370} = 5.88$, $p = .016$). There was a significant interaction between ODD/CD status and ADHD in urinary catecholamine levels (*Wilk's* λ=0.950 $F_{9,864}$ =2.054, $p = 0.031$), which was attributable to multiple simple interactions between the AD/HD-HI sub-type and the other ADHD subtypes. There was a significant main effect of ADHD subtype on urinary catecholamine levels (*Wilk's* λ=0.943, $F_{9,864}$ = 2.054, $p = 0.013$), with dopamine levels significantly increased in the AD/HD-HI group. There were no significant differences in cortisol values among the ADHD subtypes and the control group. Lower urinary epinephrine excretion, without a change in urinary cortisol excretion, may be associated with ADHD. This finding is compatible with theories of abnormal sympathetic/adrenomedulary stress response systems in ADHD.

Table of Contents

	page
Introduction	1
Evolution of Current Nosology and the Diagnostic Dilemma	1
Etiology	2
The Sympathetic Nervous System in ADHD	4
Dopamine in ADHD	5
Sympathetic and Dopaminergic Interactions	7
The HPA axis and ADHD	9
ADHD: Summary of neurochemical findings and issues	11
Statement of Purpose	12
Methods	13
Statistical Analyses	16
Results	18
Epinephrine in ADHD and ODD/CD	18
Urinary Catecholamine excretion in ADHD	19
Urinary Cortisol Excretion in ADHD	23
Discussion	25
References	29

Introduction

The syndrome now known as Attention Deficit/Hyperactivity Disorder ([1]ADHD) has been a source of much debate over the years. Discussions have concerned the diagnostic criteria, possible etiologies and the best ways to attempt to identify neurobiological alterations in ADHD. Each of these areas of discussion have raised complicated issues. Taken together, the problems have presented a serious challenge among investigators trying to understand the disorder.

Evolution of Current Nosology and the Diagnostic Dilemma

The changes in diagnostic criteria reflect the controversy regarding the range of ADHD-related behavior (Morgan et al., 1996, Lahey et al., 1984, 1985, Cantwell and Baker, 1992, Bauermeister et al., 1992, Schneider and Tan , 1997, Weinberg and Brumback, 1992. What is now known as Attention Deficit/Hyperactivity Disorder (AD/HD) has previously carried many diagnostic titles, including Strauss syndrome, Hyperkinetic Behavior Syndrome, Hyperkinetic Disorder and Minimal Brain Dysfunction. In the 1960's, the second edition of the Diagnostic and Statistical Manual (DSM II) of the American Psychiatric Association (APA, 1968) recognized the Hyperkinetic Reaction of Childhood, and this later became Attention Deficit Disorder, with and without hyperactivity (ADD/H and ADD/WO respectively) in DSM III (APA, 1980). These diagnostic categories converged in DSM III-R (APA, 1987) to form the more heterogeneous Attention Deficit Hyperactivity Disorder (ADHD) category which required eight of the fourteen symptoms listed for diagnosis. In the current DSM IV (APA, 1994) diagnostic system, a diagnosis of Attention Deficit/Hyperactivity Disorder - Inattentive Type (AD/HD-IT) requires the presence of a minimum of six of the nine inattentive symptoms for at least six months, with less than six symptoms in the

[1] ADHD is a generic term, for the purposes of this paper, referring to ADD, ADHD and AD/HD, unless otherwise specified.

hyperactive/impulsive category. The reverse is true for the diagnosis of Hyperactive-Impulsive type (AD/HD-HI. Meeting diagnostic criteria in both diagnostic categories results in the diagnosis of Combined Type (AD/HD-CT).

Co-morbidity between ADHD and many other behavioral and psychiatric disorders tends to further complicate diagnostic issues. Morgan et al (1996) noted that subjects with AD/HD-CT often have more associated conduct disorders and delinquent behaviors than those with AD/HD-IT. There were no differences between the groups regarding the internalizing disorders such as depression. These findings were replicated by Eiraldi et al.(1997), who found that those with AD/HD-CT were four times more likely to meet criteria for diagnosis of Oppositional Defiant Disorder (ODD) and six times more likely to meet diagnostic criteria for Conduct Disorder (CD) than those with AD/HD-IT. Again there were no significant differences in the prevalence of disorders of affect or mood, although there was higher prevalence of these disorders among those with ADHD than non-ADHD controls. There have been reports of co-morbidity of ADHD with learning disability and disorders of arithmetic processing (Semrud-Clikeman et al., 1992, Cantwell and Baker, 1992), and ADHD is purported to be a risk factor for adult Psychoactive Substance Use Disorders (PSUD) (Biederman et al., 1997, Horner et al, 1997).

These issues are thought to have significant impact on accuracy in diagnosing children with ADHD, and serve to compound the difficulties inherent in elucidating the etiology and pathogenesis of this disease.

Etiology

A number of studies have approached the etiology and pathogenesis of ADHD. Genetic, developmental and environmental influences have all been proposed. Investigations in this area have employed methodologies from the fields of neurochemistry and neuropharmacology, brain imaging, and genetic analyses.

Neurochemical studies have reported catecholamine metabolite abnormalities in urine, plasma, and cerebrospinal fluid (CSF) (Zametkin and Rapoport, 1987, Plitska et al., 1996). Neuropharmacologic studies have examined the effectiveness of drugs with catecholaminergic activity in treating ADHD (for eg., Sallee et al., 1992, Barkley et al., 1991, Fitzpatrick et al., 1992, Biederman et al., 1989, Conners et al., 1996, Wender et al., 1990).

Studies of brain morphology have supported theories of central catecholaminergic dysfunction in ADHD (Plitska et al., 1996). Specifically, abnormalities in perfusion (Lou et al., 1989) and size of striatal components support hypotheses of abnormal frontal and striatal dopaminergic and noradrenergic projections (Ernst and Zametkin, 1995, Castellanos, 1997). Electroencephalographic abnormalities also testify to cortical dysfunction in ADHD (Kuperman et al., 1996, Chabot et al., 1996). Varying size of regions of the corpus callosum suggests abnormal right and left brain communication, with evidence of impaired lateralized function (Baumgardner et al., 1996, Lyoo et al., 996, Garcia-Sanchez et al., 1997, Casey et al., 1997).

Genetic studies lend credence to genetic influences in ADHD (Stevenson, 1992, Sherman et al., 1997a, 1997b, Levy et al., 1997, Ernst and Zametkin, 1995), and specific associations have been made between dopamine-related genes and ADHD (Cook et al., 1995).

Although no single study has documented abnormality in an individual system that can account for the full ADHD spectrum, an alternate hypothesis implicates abnormal sympathetic and dopaminergic interactions, which affect the ability to attend to task, to sustain attention with filtering of extraneous stimuli, and to shift focus. In light of possible abnormal stress response systems in ADHD, the hypothalamic-pituitary-adrenal (HPA) axis has also been examined in children with ADHD.

The Sympathetic Nervous System in ADHD

The central sympathetic nervous system is comprised primarily of noradrenergic projections from the locus ceruleus (LC) to the ventral tegmental area (VTA) and prefrontal cortex (PFC) (Tassin, 1998). The LC also receives input from the nucleus Paragigantocellularis (PGI) in the medulla. The PGI sends excitatory input to the intermediolateral cells of the spinal cord as well, and thus affects peripheral nervous system responses (Piltska et al., 1994). Recently, it has been found that corticotrophin releasing factor (CRF) may affect the activity of many of these structures, and may play a significant role in the local stress response (Valentino et al., 1993). The interomediolateral cells of the spinal cord are thought to provide primary sympathetic and parasympathetic innervation to many organs in the body, including the adrenal medulla, the primary site for adrenaline synthesis in the periphery.

Kornetsky (1970) was first to propose a connection between increased norepinephrine and hyperactivity. Since then, attempts to document alterations in norepinephrine levels in body fluids in children with ADHD have been inconsistent (Zametkin and Rapoport, 1987), although there does seem to be some benefit to therapy that targets the proposed noradrenergic dysfunction (Arnsten et al., 1996, Biederman et al., 1989, Gualtieri et al., 1991, Conners et al., 1996, Wender et al., 1990). More recently, studies focusing on the adrenergic response have consistently suggested that this response may be blunted in children with ADHD (Klinteburg and Magnusson, 1989, Plitska et al., 1994, Girardi et al., 1995, Hanna et al., 1996b). The effectiveness of pharmacotherapy also seems to be associated with an ability to increase epinephrine (Donnelly et al., 1989, Elia et al., 1990). This may lend some credence to hypotheses of abnormal central adrenergic function (Plitska et al., 1994, Mefford and Potter, 1989, Tassin, 1998). A summary of recent studies (1987-1997) relating to the sympathetic

nervous system in ADHD is presented in Table 1. For results of studies prior to 1987, please see the review by Zametkin and Rapoport (1987).

Table 1. Studies of noradrenergic and adrenergic functioning in ADHD

	Sample Sizes				Findings in
Investigators	ADHD	Control	Fluid	Compound	ADHD
Ionescu et al., 1990	12	11	Plasma	EPI	↑ EPI
Ionescu et al., 1990	12	11	Plasma	NE	None
Plitska et al., 1994	35	22	Urine	EPI,MN	↓ EPI/MN
Plitska et al., 1994	35	22	Urine	VMA	None
Plitska et al., 1994	35	22	Urine	MHPG	None
Plitska et al., 1994	35	22	Urine	NE,NMN	↓ NE/NMN
Klinteburg, Magnusson., 1989			Urine	EPI	↓ EPI
Hanna et al., 1996a	15	16	Urine	DOPEG	↓DOPEG
Hanna et al., 1996a	15	16	Urine	NE	↓EPI
Hanna et al., 1996a	15	16	Urine	EPI	None
Hanna et al., 1996b	12	16	Urine	EPI	↓ EPI
Castellanos et al., 1994	29	20[2]	CSF	MHPG	↑ MHPG

Dopamine in ADHD

Interest in the dopaminergic model of ADHD has been prompted by the effectiveness of stimulant treatment and the development of rat models of ADHD (Shaywitz et al. , 1976, Wultz and Sagvolden, 1992, de Villiers et al., 1995, Russel et al., 1995, Kohlert et al., 1993). However, pharmacologic trials of post-synaptic

[2] This is a contrast group from a previous study by Kruesi et al., 1990

dopaminergic DA agonists have failed to show beneficial effects in the treatment of ADHD, while, at low doses, DA antagonists have been shown to be beneficial, albeit less so than is commonly seen with the stimulants (Zametkin and Rapoport 1987). A summary of current evidence suggests that dopamine may contribute to some symptoms of ADHD (Levy and Hobbes, 1988, Castellanos et al., 1994, Rogeness et al., 1989).

Centrally, dopaminergic projections originating in the VTA project to the PFC, as well as the nucleus accumbens (nAc) and other related mesolimbic structures. Abnormalities in mesolimbic and nigrostriatal dopaminergic projections have been postulated to contribute to ADHD symptomatology (Levy, 1991), and observations in neuroanatomic studies are in keeping with proposed malfunction of striatal dopaminergic neurons (Lou et al., 1989, Hynd et al., 1993, Awylard et al., 1996 and Filepek et al., 1997, Castellanos et al., 1996). In the periphery, dopamine is thought to have multiple sources, including conversion from plasma L-DOPA (Restana et al., 1994), 3-O-methyldopa (Ibarra et al., 1996) and dopamine sulfate (Ziegler et al., 1997), in addition to manufacture in sympathetic neurons and the adrenal medulla as a precursor in the synthesis of epinephrine and norepinephrine.

Previous measurements of levels of dopamine or its metabolites in urine in children with ADHD have been inconsistent (Zametkin and Rapoport, 1987). CSF studies have had slightly more consistent findings, with positive correlation made between homovanillic acid (HVA, a metabolite of dopamine) levels and indices of impulsivity and hyperactivity as well as as a predictor for response to medication (Castellanos et al., 1994, 1996, Rogeness et al., 1989).

For some time, the mesolimbic system has been thought to mediate activity levels. In rats, stimulation of the nucleus accumbens with drugs of abuse such as amphetamine and cocaine is associated with increases in locomotor activity (Kelly et al., 1975), and, indeed rat models of AD/HD have been associated with abnormalities in the nAc (Russel et al., 1995). Recently, it has also been shown that this increase in activity secondary to

stimulant activation of the nucleus accumbens can be reinforced with concurrent injection of D1 antagonists into the PFC, and can be attenuated with injection of amphetamine into the PFC, suggesting mesocortical regulation of mesolimbic dopaminergic activity (Tassin, 1998).

Cortical dopaminergic innervation is thought to perform an inhibitory function, decreasing the response to novel stimuli and focusing attention (Malone et al., 1994). Decrease in cortical DA, then, is thought to correlate with hyperarousability, hyperactivity, increased distractibility and decreased attention. The mesocortical dopaminergic system is also very sensitive to environmental stimuli, and phasic and tonic regulation of its activation via central sympathetic mechanisms may have implications in mediating attention and arousal (Tassin, 1992).

Sympathetic and Dopaminergic Interactions

Taken together, the sympathetic and dopaminergic systems may explain many of the symptoms of ADHD and therapies that affect both systems appear to be most beneficial. Some have used this observation to suggest that there is a common biochemical mediator that results in both systems being affected.

Dopamine, norepinephrine and epinephrine are synthesized from phenylalanine, through the actions of a number of enzymes, including tyrosine hydroxylase and, dopamine β hydroxylase (DβH), and phenylethanolamine-N-methyltransferase (PNMT). and metabolized by catechol-O-methyltransferase (COMT) and catecholamine oxidase (MAO) to their respective metabolites. Baker et al., (1991) found decreased levels of β-phenylethylamine (PEA), which is related to the tyrosine precursor phenylalanine, with no difference in levels of its metabolite phenylacetic acid (PAA) in 24-hour urine samples of children with ADHD compared with normal controls. They also found decreased plasma phenyethylamine and tyrosine. However, Eisenberg et al., were unable to show benefit in reduction of ADHD symptoms with tyrosine.

Others have suggested that, because of possible interrelation between these two systems, if the symptoms of ADHD are examined as if they were heterogeneous across a group of diagnostic categories instead of one diagnostic category, clearer associations may emerge. Rogeness and colleagues (1989) examined biochemical associations with symptoms corresponding to DSM III criteria for attention, impulsivity and hyperactivity as observed across diagnostic groups of children with undersocialized conduct disorder (UCD), socialized conduct disorder (CD), and a group without either diagnosis. They found that urinary levels of NE and MHPG correlated best with symptoms of inattention. Levels of vanil-mandelic acid (VMA, a metabolite of norepinephrine and epinephrine), HVA, NE and MHPG correlated with hyperactivity, while HVA and NE levels correlated with impulsivity. Correlations tended to be more evident in a group defined on the basis of low plasma DβH. When metabolites were compared across diagnostic categories, positive correlations were obtained predominantly in the undersocialized conduct disorder group, between DA, NE, VMA and EPI and hyperactivity, and between HVA and VMA and impulsivity. However, Bowden et al., (1988) found that MAO and DβH levels were lower in subjects with ADHD and UCD compared to those without UCD, and that DβH values were lower in ADHD with UCD compared to controls, suggesting that there may be a relationship between MAO and DβH levels in CD that obscures any direct relationship of these enzymes to ADHD. Stoff et al., (1989) found that increased levels of platelet MAO correlated with laboratory but not clinical measures of impulsivity, as evidenced by errors of comission on CPT testing, lending support to biochemical properties that cross diagnostic categories.

Still others have suggested that there is some interrelation between the noradrenergic and dopaminergic systems, and an abnormality in this interrelation can give rise to the symptoms and associated measured abnormalities in ADHD. In review, Tassin (1998) proposes that there may be integration of noradrenergic circuits with dopaminergic circuits in the VTA and PFC. He presents the following evidence in

support of this interaction: 1) Lesions of ascending noradrenergic projections to the VTA produced decreases in dopamine in the prefrontal cortex without affecting NE in the prefrontal cortex or DA in the nucleus accumbens. 2) There was high correlation of phasic increase in NE activity with DA activity in awake, free moving rats. 3) Lesions of ascending NE projections abolished hyperactivity and spontaneous alterations obtained by destruction of DA fibers innervating the PFC. 4) The increase in Hyperactivity induced by stimulating the nAc with amphetamine and concurrent blockade of PFC DA receptors with SCH23390, a D1 receptor antagonist, was attenuated with PFC pretreatment with prazosin, an α_1- agonist. He used these observations to suggest that NE in the VTA may maintain tonic dopaminergic projections to the PFC, while NE in the PFC may interfere with the ability of cortical DA to manipulate mesolimbic/subcortical dopaminergic activity.

While the majority of studies in ADHD tend to support abnormalities in sympathetic and dopaminergic function, some symptoms commonly associated with ADHD are comparable with those seen with impairment of the Hypothalamic-Pituitary Adrenal (HPA) Axis. The HPA system is also capable of modifying the sympathetic response. These known qualities have led to the examination of the HPA system for abnormalities in ADHD.

The HPA axis and AD/HD

The HPA axis consists of cortical and brainstem projections to hypothalamic nuclei, which then mediate pituitary release of hormonal factors which target specific end organ systems. Of particular importance in ADHD is ability of the HPA system to mediate stress responses via cortisol. The hypothalamus, serving as the primer of this response, releases corticotropin releasing factor (CRF). This stimulates the pituitary to secrete adrenocorticotropic hormone (ACTH). The target organ, the adrenal gland, then secretes cortisol and *increases sympathetic outflow*, resulting in the appropriate stress

response; the targeting of blood flow to organs that become more metabolically active as part of that response. Zametkin and Rapoport (1987) provide a brief summary of studies and findings concerning the HPA axis and ADHD.

Primary alterations in cortisol have not been found in children with ADHD. Kruesi et al. (1989) found no significant differences in urinary free cortisol output in children with ADHD, CD or matched controls. Schulz et al. (1997) found that there was no differences in plasma cortisol levels between 23 aggressive and 26 nonaggressive children with ADHD, and this result did not change when the subjects were compared depending on the presence or absence of comorbid anxiety disorders. However, without a control group for comparison, the possibility of altered plasma cortisol levels in children with ADHD relative to normal children could not be assessed.

In assessing symptom correlations with cortisol excretion, studies have documented associations between low cortisol levels and aggression (Virkunen 1985), and negative correlations were found between cortisol levels and both inattention and overactivity (Scerbo and Kolko, 1994). In the latter study, cortisol levels were positively correlated with parent-rated internalizing behaviors, consistent with their hypothesis that high basal cortisol levels may correlate with internalizing behaviors. Tennes et al., (1986) found that increased levels of urinary cortisol were associated with increased social interaction which is not in agreement with the previously mentioned studies, but is consistent with previous work done by others (see Tennes et al., 1986).

The neurotransmitters DA and 5-Hydroxytryptamine (5-HT) are known to influence the hypothalamic and pituitary functioning. For example, dopamine exerts an inhibitory effect on the secretion of prolactin (Seri, 1995), and some studies have used prolactin measurements to document the effect of stimulants (Sallee et al., 1992). In their model, Peled et al (1997) suggest that ADHD is a result of dysregulation of hypothalamic setpoints, secondary to prenatal or childhood stress, which causes oscillation between states of autonomic oversensitivity and hyperarousability, and those of blunted

responsivity. In keeping with this model, one would expect heightened baseline HPA activity (reflected in levels of ACTH and cortisol) and/or responsivity in subjects displaying more externalizing symptoms and lower activity or responsivity associated with more internalizing symptoms.

ADHD: Summary of neurochemical findings and issues

So far, the most consistent findings lend at least indirect support to major proposals of central dopaminergic and sympathetic dysfunction in ADHD, and some have tried at length to document their peripheral connections (review by Goldstein, 1998, Yanagihara et al., 1998). Studies in ADHD have been very dissimilar in subject selection and methodologies employed, and it is suspected that some of their inherent differences may contribute to the inconsistencies in neurobiological observations in ADHD. A particularly consistent finding, however, has been the association of blunted epinephrine excretion with ADHD.

It is still unclear whether there are measurable catecholaminergic abnormalities in body fluids of children with ADHD. Sample size, test conditions, type of sample collected and method, as well as diagnostic categorization without careful attention to comorbid disorders, may account for many of the variations seen in the data. A large, carefully diagnosed sample, with well defined test conditions and sample collection techniques, may serve to clarify these issues. Specifically, whether group mean differences exist between children with and without ADHD, or between ADHD subtypes, on the basis of urinary levels of EPI, NE, and DA. It would also be important to assess whether observed differences can be explained by existing comorbid diagnoses, particularly Conduct Disorder, Oppositional Defiant Disorder, Reading or Math Disorders, or any combination of the above.

Few studies have examined the effects of comorbid diagnoses such as behavior disorders (ODD/CD) or learning disorders (RD/MD) on urinary catecholamine levels. In

one study which examined 24 hour urine collections, there was a report of increased HVA and epinephrine excretion in subjects with ODD/CD, compared with normal controls (Oades et al., 1994). It would be helpful to replicate these findings in relation to epinephrine, as well as to examine the catecholamines in ODD/CD directly, as from these relationships we may be able to comment on the effect of having ODD/CD as comorbid diagnoses in children with AD/HD in terms of urinary catecholamine excretion.

Abnormalities in cortisol levels have not been found in children with ADHD ,although cortisol levels have been correlated with increased activity and high levels with inattention and other internalizing behaviors. Because of the known effects of the catecholamines on the HPA axis, some of the abnormalities observed in the HPA system may reflect abnormal neurotransmitter function, and may correct when treatment of the specific abnormality commences. There is therefore a need to compare cortisol levels in children with ADHD relative to control groups, and further study of the effect of treatment on cortisol is also warranted. Finally, the possibility of a blunted stress response in children with ADHD suggests that an examination of stress-induced cortisol response in children with ADHD would be informative.

Statement of Purpose

Our aims are:

1. To attempt to replicate previous findings of reduced epinephrine excretion in children with ADHD.

2. To attempt to replicate previous findings of increased urinary epinephrine excretion in children with ODD/CD.

3. To assess possible abnormal interrelations between the catecholamines and cortisol in children with different sub-types of ADHD, controlling for comorbid learning (RD and MD) and behavioral (ODD and CD) disorders.

Method

Subjects

Participants in this study were boys (N = 373) between 7.5 and 13.5years of age, who were recruited from the community by referral from schools, parents, professionals, and local media announcements over a period of five years. Male controls were recruited over the same period using newspaper advertisements, letters to schools, and posters in local stores and libraries requesting the participation of boys without problems of learning or attention. Entry criteria based on telephone screening and a best estimate diagnosis included age, gender, no history of emotional problems or neurologic disorder, WISC-R (Wechsler, 1974) full scale IQ 80, English as a native language, normal or corrected vision and hearing, and abstinence from all medications (except Acetaminophen or antibiotics) for at least seven days prior to enrollment. Also, siblings were excluded from the study population. The best estimate diagnosis was derived from the Yale Children's Inventory, a questionnaire formulated for parents and used to assess whether the child met behavioral criteria for ADHD, the Swanson, Nolan and Pelham checklist (SNAP), and the Diagnostic Interview Schedule for Children (DISC-R) (Shaffer et al., 1996). The final diagnostic categories were determined using the DISC-R (2.3, Shaffer, Fisher et al., 1996), as the primary diagnostic tool. The DISC-R is a structured interview of the proband's parent (usually the mother) to assess the child's psychiatric diagnostic status. The current version was formatted based on the newly released strict DSM IV criteria, and thus required meeting the same symptom requirements upon interview as the current DSM IV. Children were also assessed for DSM-IV defined ODD and CD. Learning disabilities in reading and math were assessed using low achievement and discrepancy criteria on the Woodcock-Johnson Psychoeducational battery (Woodcock, 1978). For reading disability (RD), the child's average age-adjusted standard score for two subtests (Letter-Word Identification and Word Attack) served as the criterion and for Math Disability (MD), age-adjusted standard score for the Calculation subtest served as the criterion. Achievement scores in the lowest quartile (age-standard score < 90) in

reading or math met low achievement criteria. Discrepancy criteria were established using a regression equations between the achievement criteria and WISC-R Full Scale IQ (FSIQ) derived from a normative population.

Final groups were established by DISC 2.3 diagnosis, and included normal controls, AD/HD-IT, AD/HD-HI, or AD/HD-CT. The groups were subdivided separately to assess for effects of comorbid learning disorder, or ODD/CD. The distribution of subjects in the diagnostic groups is presented in Table 2.

Table 2. Distribution of Subjects by Diagnostic Categories

	Subjects Without OD/CD				
	Normal	AD/HD-IT	AD/HD-HI	AD/HD-CT	Total
No LD	30	19	6	15	70
LD	28	21	4	9	62
	Subjects With OD/CD				
No LD	18	27	13	71	129
LD	12	20	9	71	112

Sample Collection

Urine was collected between the hours of nine a.m. and twelve noon, generally on the first day of testing. The procedure was explained to the children. Voiding was accomplished before the beginning of collection. The children were encouraged to drink several glasses of water during the collection period. In the event of test interference or insufficient volume, urine was re-collected on the second day of testing. These samples were maintained separate from those obtained on Day 1. Total urine volume was measured, and 5 ml of each sample was placed in storage vials provided by the Child Study laboratory. The storage vials contained 33 µl of 5% sodium metabisulfate preservative, 33 µl of EDTA, and 20 µl of glacial acetic acid. Upon receiving the

samples from the test site, 3 ml of urine was removed, and to the remainder 6 µl of the internal standard (HPLC methods) DHBA was added, along with an additional 20 µl of sodium metabisulfate. The samples were then stored at -70°C until the time of analysis.

Testing

Four groups of tests, each consisting of three or more tests. were administered during urine collection. The first group included assessments of anxiety and visio-spatial and motor co-ordination. These were administered at the beginning of sample collection. The other three groups included assessments of memory and attention, with focused and divided attention being assessed.

Urine Analyses

Urine was analyzed for concentration of DA, NE , EPI and cortisol. Urinary NE, DA and EPI were measured using high pressure liquid chromatography flurometry (Anderson et al., 1988). Urine samples were thawed, and the pH was adjusted to 8.4-8.7 by the addition of 3 M of pH 8.7 TRIS buffer. Fifty milligrams of alumina was then added, and the samples were mixed on a rotary mixer for 10 minuites. The supernantant was then discarded and, after washing twice with distilled water, 250 µl of 1.0 molar acetic acid was added to the alumina to elute the catecholamines. The supernatant eluant was removed and the samples were then stored in 1.5 ml polypropylene tubes for HPLC analysis. Twenty to 50 µl of each solution was injected into the chromatographic system. at a flow rate of 2.0 ml/min. For flourometric detection, the excitation wavelength was 285 ηm, and emission wavelength (with minimal background interference) was 305 ηm. Standards were injected every 8-10 samples. Concentration (ηg/ml) of the respective catecholamine was calculated based on the known concentration (200 ηg/ml) of the internal standard DHBA (Aldrich Chemical Co., Milwaukee, WI 53233), the response ratios of the urine standards and the sample peak ratios. Cortisol was measured using

coated tube radioimmunoassay kits purchased from Diagnostic Systems Laboratory (Webster, TX). The kit included rabbit anti-cortisol antibody-labeled tubes, calibration standards for urine, high and low samples, and radioactive I-125 labeled cortisol in sodium azide preserved buffer.

Statistical Analysis

Observed excretion rates for NE, EPI, DA, and cortisol were expressed as ng/hr/m^2. Body surface area adjustments were made using Mosteller's equation (Briars and Bailey, 1994).

Epinephrine in ADHD and OD/CD

Two groups were identified: those who did not meet criteria for ADHD (normal), and those who met any ADHD subtype criteria (ADHD). These groups were then subdivided into those who met OD/CD criteria and those who did not. A univariate two-way ANOVA was performed to determine the effects of ADHD status, OD/CD status, and their interaction on urinary epinephrine excretion.

Urinary catecholamine and cortisol excretion in subtypes of ADHD

Before proceeding with inferential statistics, the relationships among the dependent measures (body-surface-area adjusted urinary excretion rates of NE, EPI, DA, and cortisol) presented in Table 3 were estimated using Pearson product-moment correlations. As can be seen in the table, all three catecholamines are moderately intercorrelated and cortisol is correlated to an even lesser extent with any of the

Table 3. Pearson Correlations Among Body-Surface-Area Adjusted Catecholamines and Cortisol

	EPI	DA	Cortisol
NE	.53	.70	.29
EPI		.52	.26
DA			.37

Given the relative strengths of the interrelationships among the dependent measures, we chose to analyze the three intercorrelated catecholamines in a multivariate model and to analyze cortisol in a separate univariate model. We tested for the separate and joint effects of AD/HD status, LD status, and OD/CD status using a three-factor design. The AD/HD factor comprises four levels, and the OD/CD factor and LD factor each comprise two levels.

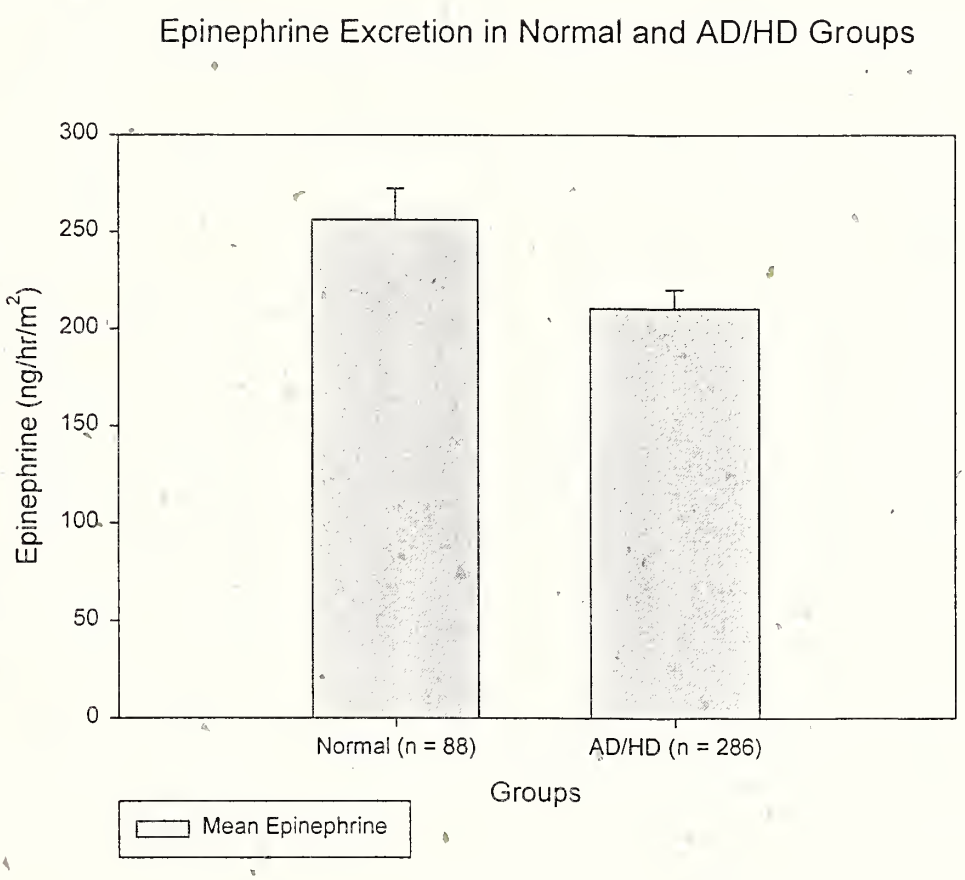
Means and standard errors for the dependent measures were calculated. The three catecholamines were analyzed using a three-way MANOVA; cortisol was analyzed in a three-way ANOVA. Omnibus tests of main effects and two- and three-way interactions were tested at $p < .05$ level. Post-hoc comparisons were tested at p values Bonferroni-adjusted for the number of contrasts tested within each effect to maintain an effect-wise $p < .05$. For the MANOVA, omnibus and post-hoc comparisons were interpreted with canonical correlations.

Results

Epinephrine and ADHD and OD/CD

There was no statistically significant interaction between ADHD and OD/CD status, nor was there a significant main effect of OD/CD status on urinary epinephrine excretion. There was, however, a significant main effect of ADHD status ($F_{1,370} = 5.88$, $p = .016$), with ADHD being associated with lower urinary epinephrine excretion. Body-surface-area adjusted mean epinephrine values and standard errors obtained for the normal group and those with ADHD are presented in Figure 1.

Figure 1.



Urinary Catecholamine Excretion in ADHD

Means and standard errors of the body-surface-area adjusted excretion rates for norepinephrine, epinephrine, and dopamine in the ADHD groups and OD/CD status groups are presented in Table 4. The MANOVA summary is presented in Table 5.

Table 4. Means and Standard Errors of Norepinephrine, Epinephrine, and Dopamine Excretion by AD/HD Group and OD/CD Status

		Group				
Group		Normal	ADHD-IT	ADHD-HI	ADHD-CT	Total
No OD/CD	n	58	40	10	24	132
	NE	1007±56	875±68	883±139	1013±90	944±47
	EPI	254±19	200±23	209±46	201±30	216±16
	DA	7379±398	6767±480	9568±979	6643±639	7589±331
OD/CD	n	30	47	22	142	241
	NE	1052±80	879±63	1001±93	964±36	974±36
	EPI	257±27	203±21	272±31	222±12	238±12
	DA	8058±565	6865±447	7494±658	7445±255	7466±252
Total	n	88	87	32	166	
	NE	1030±49	877±46	942±83	989±49	
	EPI	255±16	201±16	241±28	211±16	
	DA	7719±346	6816±328	8531±590	7044±344	

The three-way interaction is not statistically significant. There is, however, a significant (p = .031) two-way ADHD X OD/CD interaction and a trend (p = .052) toward a two-way OD/CD X LD interaction. Finally, there is a significant main effect for ADHD group.

Table 5. MANOVA Summary Table for Norepinephrine, Epinephrine, and Dopamine

Source	*df*	Wilk's λ	*F*	*p*
AD/HD	9,864	.943	2.337	.013
OD/CD	3,355	.992	.927	ns
LD	3,355	.994	.665	ns
AD/HD X OD/CD	9,864	.950	2.054	.031
AD/HD X LD	9,864	.988	.466	ns
OD/CD X LD	3,355	.979	2.594	.052
AD/HD X OD/CD X LD	9,864	.984	.651	ns

To interpret the significant two-way interaction between ADHD and OD/CD, tests of pairwise simple interactions were conducted. These simple interactions compared the differences between those without comorbid OD/CD and those with comorbid OD/CD from each pair of ADHD groups. The probabilities for these comparisons are presented in Table 6.

Table 6. Summary of Probabilities for MANOVA ADHD X OD/CD: Simple Interaction Comparisons

Groups Contrasted		*p*	Canonical Variate Loadings		
			NE	EPI	DA
AD/HD - IT	Normal	ns			
AD/HD - HI	Normal	.005*	.104	.250	-.553
AD/HD - CT	Normal	ns			
AD/HD - HI	AD/HD - IT	.015	.183	.284	-.492
AD/HD - CT	AD/HD - IT	ns			
AD/HD - CT	AD/HD - HI	.001*	.211	.156	-.516

Criterion for effect-wise $p < .05$ after Bonferroni adjustment is $p < .0083$

Statistically significant simple interactions were detected for two of six comparisons. A third comparison approached, but did not reach, statistical significance. Each of those three comparisons included the AD/HD - HI group. The canonical variate loadings for the three contrasts indicate that DA contributes most to the statistically significant interactions.

The significant two-way ADHD X OD/CD interaction may be interpreted in terms of differences between those with and without comorbid OD/CD within each ADHD group. A summary of these tests of simple main effects of OD/CD status within each ADHD group is presented in Table 7. A statistically significant difference was found between those with and without comorbid OD/CD in the AD/HD - HI group. Inspection of the canonical variate loadings in Table 7 and the means in Table 4 reveals that the No OD/CD subjects in the AD/HD – HI group exhibit a high level of DA (contributing most to the significant difference) and secondarily, a low level of EPI (contributing slightly to the significant difference) relative to the OD/CD subjects in the AD/HD -HI group.

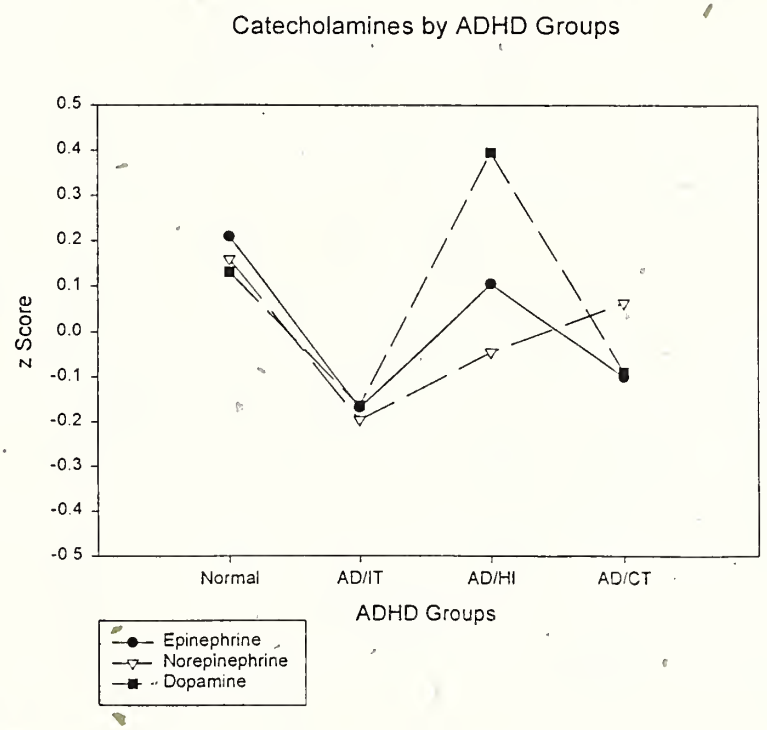
Table 7. Summary of MANOVA Simple Main Effects of ODCD Within Each ADHD Group

		Canonical Variate Loadings		
Group	*p*	NE	EPI	DA
Normal	ns			
AD/HD - IT	ns			
AD/HD - HI	.004*	.193	.304	-.487
AD/HD - CT	ns			

* Criterion for effect-wise $p < .05$ after Bonferroni adjustment is $p < .0125$

To interpret the significant MANOVA main effect for ADHD group, the means for NE, EPI, and DA were normalized (converted to the z - distribution) and the resultant plot of group means is presented in Figure 2.

Figure 2.



The high mean DA for the AD/HD - HI group is most pronounced. To interpret the main effect statistically, pairwise comparisons for the three catecholamines were calculated. Results of those comparisons are presented in Table 8. As would be expected from the analyses of the significant two-way interaction, a statistically significant difference was found between AD/HD -HI and AD/HD - CT groups. Furthermore, three other comparisons exhibited trends toward statistical significance: AD/HD - IT verses Normal, AD/HD - HI verses Normal, and AD/HD - HI verses AD/HD - IT.

Table 8. Summary of Probabilities for ADHD Group Pairwise Comparisons

			Canonical Variate Loadings		
Groups Contrasted		*p*	NE	EPI	DA
AD/HD - IT	Normal	.070			
AD/HD - HI	Normal	.053			
AD/HD - CT	Normal	ns			
AD/HD - HI	AD/HD - IT	.032			
AD/HD - CT	AD/HD - IT	ns			
AD/HD - CT	AD/HD - HI	.005*	-.135	.252	.606

* Criterion for effect-wise $p < .05$ after Bonferroni adjustment is $p < .0083$

Urinary Cortisol Excretion in ADHD

Means and standard errors of the body-surface-area adjusted cortisol for the ADHD groups and OD/CD status groups are presented in Table 9 and the ANOVA summary table is presented in Table 10. There are no statistically significant interactions or main effects. There is, however, a trend for the main effect of OD/CD status ($F_{1,354} = 2.63$, $p = .106$). As can be seen in Table 9, the observed means for the four groups with comorbid OD/CD are systematically higher than the corresponding means for the groups without comorbid OD/CD.

Table 9. Means and Standard Errors of Cortisol by ADHD Group and OD/CD Status

		AD/HD Group				
Group		Normal	AD/HD -IT	AD/HD –HI	AD/HD -CT	Overall
No OD/CD	n	52	33	10	23	118
		1467±160	1433±202	1104±373	1417±253	1355±130
OD/CD	n	30	43	20	130	223
		1810±215	1712±178	1516±259	1440±101	1620±99
Overall	n	82	76	30	153	
		1639±134	1573±135	1310±227	1428±136	

Table 10. ANOVA Summary for Cortisol

Source	*df*	*F*	*p*
ADHD	3	.748	ns
OD/CD	1	2.630	.106
LD	1	.650	ns
ADHD X OD/CD	3	.316	ns
ADHD X LD	3	.291	ns
ODCD X LD	3	.175	ns
ADHD X OD/CD X LD	3	.289	ns
Error	354	(1332794.5)	

Note. Number in parentheses is a mean-squared error.

ns: $p \geq 0.05$

Discussion

Our results are consistent with previous reports of reduced urinary epinephrine excretion in ADHD (Klinteberg and Magnusson, 1989, Hanna et al., 1996b). This suggests that there are differences in urinary epinephrine excretion associated with ADHD. We are confident in the validity of this finding as, to date, this is the largest study of catecholamine excretion in children with ADHD. While it is unclear to what extent comorbid disorders were excluded in the previous studies, comorbid OD/CD did not significantly impact on urinary epinephrine excretion in this study.

We were unable to replicate previous findings of increased urinary epinephrine excretion in association with OD/CD (Oades et al., 1994), although both the control group and those with ADHD tended to have higher epinephrine levels in association with OD/CD. Although there are other conditions that tend to co-exist with ADHD, particularly learning and affective disorders, previous reports show no effect of the presence of comorbid affective disorder on urinary catecholamines in ADHD (Morgan et al., 1996, Eiraldi et al., 1997), and our studies have shown that there was no significant interaction between learning disorder and ADHD diagnostic category.

Previously thought to be derived solely from the adrenal gland, studies now have demonstrated other sources of urinary epinephrine. Kennedy et al.(1995) have demonstrated the existence of phenylethanolamine N-methyltransferase (PNMT), the enzyme which converts norepinephrine to epinephrine, as well as nonspecific N-methyl transferase (NMT), which can convert dopamine and other phenyethanolamines to epinephrine precursors, in many other tissues, including human lung, kidney, heart, pancreas, liver and spleen. In their studies, they obtained positive correlation between the presence of PNMT and epinephrine secretion within a particular tissue type.

Although it is still thought that the majority of urinary epinephrine is derived from filtered plasma epinephrine, increasing evidence continues to support the existence of other sources for urinary epinephrine. Ziegler et al. (1997) infused ^{3}H-Epi in humans to

determine what fraction of urinary epinephrine is filtered from plasma. From estimates of expected clearance vs. observed clearance of epinephrine, it was determined that almost half of urinary epinephrine is obtained from sources other than the adrenal. The authors hypothesized that other sources might include neuronal release in the kidney (though this is expected to be miniscule), as well as conversion of sulfated plasma epinephrine back to epinephrine by kidney sulfatases, conversion of norepinephrine to epinephrine in the kidney by PNMT, conversion of other methyl amines into epinine by NMT, which can then be converted to epinephrine, and conversion of dopamine sulfate (from plasma) into epinephrine.

The catecholamines are known to have multiple peripheral effects in the body, and in particular, they direct the body's response to stress. Epinephrine and norepinephrine increase heart rate, and serve primarily to direct blood flow to the most needed areas in the *flight or fight* response. By stimulation of α_1 receptors, they cause peripheral vasoconstriction, while by stimulating β_1 and β_2 receptors, they cause increased heart rate and smooth muscle dilitation, respectively. The geographic location of adrenergic receptor subtypes in the body allow for a preferential shuttling of oxygenated blood to responding organs (such as skeletal muscle) and away from nonresponding organs (such as skin) during the stress response.

The finding of depressed epinephrine response to stress in children with ADHD compared with normal children may indicate that the type of stressor may have been ineffective at stimulating epinephrine release. This would imply that stress responses in ADHD require more robust stimulation than they do in normal children, representing either an impairment in the elicitation of the sympathetic/adrenomedullary system in AD/HD, or an intrinsic deficiency in the system itself. Such impairment could occur as a result of decreased ability to generate epinephrine, either from norepinephrine, or from other known sources of urinary epinephrine, but could not reflect total adrenomedullary dysfunction, as there was no impact on norepinephrine levels (although it is possible that

norepinephrine secretion was maintained by conversion of dopamine). Yet another explanation would be that this is a reflection of a centrally mediated change in the stress response in children with AD/HD. This is a most attractive hypothesis, since it would not only account for findings related to epinephrine, but to other catecholamines as well. Within the design of our study, however, it is also the most difficult to prove, since we did not directly measure any central components. Whether of central or peripheral origin, this finding does suggest that there is an abnormality within the stress response systems of those with AD/HD, which manifest themselevs in lower epinephrine excretion rates.

In addition, we found that it may be possible to distinguish the AD/HD-HI sub-type from other AD/HD groups as well as from normal children on the basis of urinary dopamine levels, as they tended to have higher levels of urinary dopamine than other AD/HD groups or normal controls. Sources of urinary dopamine include plasma L-dopa (Restana et al., 1994), 3-*O*-methyldopa (Ibarra et al., 1996) and dopamine sulfate (Ziegler et al., 1997), in addition to dopamine manufactured as a precursor in the synthesis of norepinephrine and epinephrine. Dopamine has multiple peripheral effects, which are reflected by the anatomic locations of peripheral dopaminergic receptors, but it also extends its peripheral effects to cause feedback inhibition of some central nervous system components (Seri, 1995). An increase in urinary dopamine, therefore, may be explained by decreased peripheral conversion to its successors in the metabolic pathway (NE and EPI) or to its metabolites. The fact that this effect is isolated to the AD/HD-HI sub-type, while, for example, epinephrine levels are depressed in all three AD/HD diagnostic categories, makes this less likely. An explanation involving central sympathetic abnormality may explain this finding in the light of known interactions between prefrontal cortical noradrenergic and cortical and subcortical dopamine and relationships between dopamine and hyperactivity (Tassin, 1998). It is also possible that AD/HD-HI may represent a separate diagnostic entity, particularly in the light of the unique observed interaction between epinephrine and dopamine in children with AD/HD-HI and OD/CD.

Alternately, a TYPE I error occurred based on the small sample size. Further examination is warranted, therefore, before firm theories can be developed.

Cortisol did not serve to distinguish between ADHD subtypes or to distinguish those with ADHD from those without ADHD. This is also in agreement with previous findings (Kruesi et al., 1989, Shulz et al., 1997), and may suggest that the abnormalities seen in ADHD may not involve the HPA axis directly, but its involvement may be contingent upon its ability to affect the symathetic stress response systems.

In summary, the major finding of our study was lower urinary excretion of epinephrine during the stress of cognitive testing in ADHD versus normal children. This result replicates previous findings of several, substantially smaller, studies, and suggests that the adrenomedullary response to stress is altered in ADHD children. In contrast, group differences were not seen for urinary indices of sympathetic neuronal activity, or HPA axis functioning. The results of our study, together with previous research in this area, indicate that further investigation targeting peripheral (adrenomedullary) and central epinephrine systems is warranted. Such research may provide a basis for subtyping ADHD patients, and may lead to the development of new pharmacological approaches to the treatment of ADHD.

References

American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 2nd Edition. Washington, DC: American psychiatric Association, 1968.

American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition. Washington, DC: American Psychiatric Association, 1980.

American Psychiatric Association: Diagnostic and Statistican Manual of Mental Disorders, 3rd Edition-Revised. Washington, DC: American Psychiatric Association, 1987.

American Psychiatric Association: Diagnostic and Statistcial Manual of Mental Disorders, 4th Edition. Washington, DC: American Psychiatric Association, 1994.

Anderson GM, Durkin TA, Morton JB, Cohen DJ: Liquid chromatographic determination of urinary catecholamines after one-step alumina extraction. J Chromatography 424: 373-377, 1988.

Arnsten AFT, Steere JC, Hunt RD: The Contribution of α_2-Noradrenergic Mechanisms to Prefrontal Cortical Cognitive Function. (Potential Significance for Attention-Deficit Hyperactivity Disorder.) Arch Gen Psychiatry 53: 448-455, 1996.

Aylward, EH, Reiss, AL, Reader, MJ, Singer, HS, Brown, JE, Denckla MB: Basal Ganglia Volumes in Children with Attention-Deficit Hyperactivity Disorder. J Child Neur 11: 112-115, 1996.

Baker GB, Bornstein RA, Rouget AC, Ashton SE, van Muyden JC, Coutts RT: Phenylethylaminergic Mechanisms in Attention-Deficit Disorder. Biol Psychiatry 29: 15-22, 1991.

Barkley RA, DuPaul GJ, McMurray MB: Attention Deficit Disorder With and Without Hyperactivity: Clinical Response to Three Dose Levels of Methylphenidate. Pediatrics 87: 519-531, 1991.

Bauermeister JJ, Alegría M, Bird HR, Rubio-Stpiec M, Canino G: Are Attentional-Hyperactivity Deficits Unidimentional or Multidimensional Syndromes? Empirical Findings from a Community Survey. J Am Acad Child Adolesc Psychiatry 31: 423-431, 1992.

Baumgardner TL, Singer HS, Denckla MB, Rubin MA, Abrams MT, Colli MJ, Reiss AL: Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. Neurology 47: 477-482, 1996.

Biederman J, Baldessarini RJ, Wright V, Knee D, Harmatz JS: A Double-Blind Placebo Controlled Study of Desipramine in the Treatment of ADD: I. Efficacy. J Am Acad Child Adolesc Psychiatry 28: 777-784, 1989.

Biederman J, Wilens T, Mick E, Faraone S, Weber W, Curtis S, Thornell A, Pfister K, Garcia Jetton J, Soriano J: Is ADHD a Risk Factor for Psychoactive Substance Use Disorders? Findings From a Four-Year Prospective Follow-Up Study. J Am Acad Child Adoloesc Psychiatry 36: 21-29, 1997.

Bowden CL, Deutsch CK, Swanson JM: Plasma Dopamine-ß-Hydroxylase and Platelet Catecholamine Oxidase in Attention Deficit Disorder and Conduct Disorder. J Am Acad Child Adolesc Psychiatry 27: 171-174, 1988.

Briars GL, Bailey BJ: Surface area estimation: pocket calculator v nomogram. Archives of Disease in Chidhood 70: 246-247, 1994.

Cantwell DP, Baker L: Attention Deficit Disorder with and without Hyperactivity: A Review and Comparison of Matched Groups. J Am Acad Child Adolesc Psychiatry 31: 432-438, 1992.

Casey BJ, Castellanos FX, Giedd JN, Marsh WL, Hamburger SD, Schubert AB, Vauss YC, Vaituzis AC, Dickstein DP, Sarfatti SE, Rapoport JL: Implication of Right Frontostriatal Circuitry in Response Inhibition and Attention-Deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatry 36: 374-383, 1997.

Castellanos FX, Elia J, Kruesi MJP, Gulotta CS, Mefford IN, Potter WZ, Ritchie GF, Rapoport JL: Cerebrospinal Fluid Catecholamine Metabolites in Boys With Attention-Deficit Hyperactivity Disorder. Psychiatry Res 52: 305-316, 1994.

Castellanos FX, Geidd JN, Marsh WL, Hamburger SD, Vaituzis AC, Dickstein DP, Sarfatti SE, Vauss YC, Snell JW, Lange N, Kaysen D, Krain AL, Ritchie GF, Rajapakse JC, Rapoport JL: Quantitative Brain Magnetic Resonance Imaging in Attention-Deficit Hyperactivity Disorder. Arch Gen Psychiatry 53: 607-616, 1996

Castellanos FX, Elia J, Kruesi MJP, Marsh WL, Gulotta CS, Potter WZ, Ritchie GF, Hamburger SD, Rapoport JL: Cerebrospinal Fluid Homovanillic Acid Predicts

Behavioral Response to Stimulants in 45 Boys with Attention Deficit/Hyperactivity Disorder. Neuropsychopharm 14: 125-137, 1996.

Castellanos FX: Toward a Pathophysiology of Attention Deficit/Hyperactivity Disorder. Clinical Pediatrics: 381-390, 1997.

Chabot RJ, Serfontein G: Quantitative Electroencephalographic Profiles of Children with Attention Deficit Disorder. Biol Psychiatry 40: 951-963, 1996.

Conners KC, Casat CD, Gualtieri CT, Weller E, Reader M, Reiss A, Weller RA, Khayrallah M, Ascher J: Buproprion Hydrochloride in Attention Deficit Disorder with Hyperactivity. J Am Acad Child Adolesc Psychiatry 35: 1314-1321, 1996.

Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Leventhal BL: Association of Attention-Deficit Disorder and the Dopamine Transporter Gene. Am J Hum Genet 56: 993-998, 1995.

Donnelly M, Rapoport JL, Potter WZ, Oliver J, Keysor CS, Murphy DL: Fenfluramine and Dextroamphetamine Treatment of Childhood Hyperactivity. Arch Gen Psychiatry 46: 205-212, 1989.

Eiraldi RB, Power TJ, Maguth Nezu C: Patterns of Comorbidity Associated With Subtypes of Attention-Deficit/Hyperactivity Disorder Among 6- to 12-Year-Old Children. J Am Acad Child Adolesc Psychiatry 36: 503-514, 1997.

Elia J, Borcherding BG, Potter WZ, Mefford IN, Rapoport JL, Keysor CS: Stimulant drug treatment of hyperactivity: biochemical correlates. Clin Pharm & Therapeutics 48: 57-66, 1990.

Ernst M, Zametkin A: The Interface of Genetics, Neuroimaging and Neurochemistry in Attention-Deficit Hyperactivity Disorder. Psychopharmacology: The Fourth Generation of Progress. Bloom and Kupfer, Raven Press Ltd., New York, 1995.

Filipek, P.A., Semrud-Clikeman, M., Steingard, R.J., Renshaw, P.F., Kennedy DN, Beiderman J: Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. Neurology 48: 589-601, 1997.

Fitzpatrick PA, Klorman R, Brumaghim JT, Borgstedt AD: Effects of Sustained-Release and Standard Preparations of Methylphenidate on Attention Deficit Disorder. J Am Acad Child Adoloesc Psychiatry 31: 226-234, 1992.

García-Sánchez C, Estévez-González A, Suárez-Rmero E, Junqué C: Right Hemisphere Dysfunction in Subjects With Attention-Deficit Disorder With and Without Hyperactivity. J Child Neurol 12: 107-115, 1997.

Girardi NL, Shaywitz SE, Shaywitz BA, Marchione K, Fleischman SJ, Jones TW, Tamborlane WV: Blunted Catecholamine Responses after Glucose Ingestion in Children with Attention Deficit Disorder. Pediatr Res 38: 539-542, 1995.

Goldstein DS: Catecholamines In The Periphery. Advances in Pharmacology 42: 529-539, 1998.

Gualtieri CT, Keenan PA, Chandler M: Clinical and Neuropsychological Effects of Desipramine in Children with Attention Deficit Hyperactivity Disorder. J Clin Psychopharmacol 11: 155-159, 1991.

Hanna GL, Ornitz EM, Harihan M: Urinary catecholamine excretion and behavioral differences in ADHD and normal boys. J Child Adolesc. Psychopharmacol. 6: 63-73, 1996a.

Hanna GL, Ornitz EM, Harihan M: Urinary Epinephrine Excretion During Intelligence Testing in Attention-Deficit Hyperactivity Disorder and Normal Boys. Biol Psychiatry 40: 553-555, 1996b.

Horner BR, Scheibe KE: Prevalence and Implications of Attention-Deficit Hyperactivity Disorder Among Adolescents in Treatment for Substance Abuse. J Am Acad Child Adolesc Psychiatry 36: 30-36, 1997.

Hynd, G.W., Hern, K.L., Novey, E.S., Eliopulos, D., Marshall, R., Gonzalez, J.J., Voeller, K.K: Attention Deficit-Hyperactivity Disorder and Asymmetry of the Caudate Nucleus. J Child Neurol 8: 339-346, 1993.

Ibarra FR, Aguirre J, Nowicki S, Barontini M, Arrizurieta EE, Armando I: Demethylationof 3-*O*-methyldopa in the kidney: a possible source for dopamine in urine. Am J. Physiology 270 (5 Pt 2): F862-868, 1996.

Ionescu G, Kiehl R, Ona L, Wichmann-Kunz F: Abnormal Plasma Catecholamines in Hyperkinetic Children. Biol Psychiatry 28: 547-550, 1990.

Kennedy B, Bigby TD, Ziegler MG: Nonadrenal Epinephrine-forming Enzymes in Humans. Characteristics, Distribution, Regulation and Relationship to Epinephrine levels. J. Clinical Investigation 95: 2896-2902, 1995.

Kelly PH, Seviour PW, Iverson SD: Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research 94: 507-522, 1975.

Klinteberg BA, Magnusson D: Aggressiveness and hyperactive behavior is related to adrenaline excretion. European J. Pers. 3: 81-93, 1989.

Kohlert JG, Bloch GJ: A Rat Model For Attention Deficit-Hyperactivity Disorder. Physiol and Behavior 53: 1215-1218, 1993.

Kornetsky C: Psychoactive drugs in the immature organism. Psychopharmacologia 17: 105-136, 1970.

Kruesi MJ, Schmidt ME, Donnelly M, Hibbs ED, Hamburger SD: Urinary free cortisol output and disruptive behavior in children. J Am Acad Child Adolesc Psychiatry 28: 441-443, 1989.

Kruesi MJP, Rapoport JL, Hamburger S, Hibbs E, Potter WZ, Lenane M, Brown GL: Cerebrospinal Fluid Catecholamine Metabolites, Aggression, and Impulsivity in Disruptive Behavior Disorders of Children and Adolescents. Arch Gen Psychiatry 47: 419-426, 1990.

Kuperman S, Johnson B, Arndt S, Lindgren S, Wolraich M: Quantitative EEG Differences in an Nonclinical Sample of Children with ADHD and Undifferentiated ADD. J Am Acad Child Adolesc Psychiatry 35: 1009-1017, 1996.

Lahey BB, Schaughency EA, Strauss CC, Frame CL: Are attention deficit disorders with and without hyperactivity similar or dissimilar disorders? J Am Acad Child Adolesc Psychiatry 23:302-309, 1984.

Lahey BB, Schaughency EA, Frame EL, Strauss CC: Teacher ratings of attentional problems in children experimentally classified as exhibiting attention deficit disorders with and without hyperactivity. J Am Acad Child Adolesc Psychiatry 24: 613-616, 1985.

Levy F, Hobbes G: The Action of Stimulant Medication in Attention Deficit Disorder with Hyperactivity: Dopaminergic, Noradrenergic, or Both? J Am Acad Child Adoloesc Psychiatry 27: 802-805, 1988.

Levy F: The Dopamine Theory of Attention Deficit Hyperactivity Disorder (ADHD). Aus New Zeal J Psychiatry 25: 277-283, 1991.

Levy F, Hay DA, McStephen M, Wood C, Waldman I: Attention-Deficit Hyperactivity Disorder: A Category or a Continuum? Genetic Analysis of a Large-Scale Twin Study. J Am Acad Child Adolesc Psychiatry 36: 737-743, 1997.

Lou HC, Henriksen L, Bruhn P, Børner H, Nielson JB: Striatal Dysfunction in Attention Deficit and Hyperkinetic Disorder. Arch Neurology 46: 48-52, 1989.

Lyoo IK, Noam GG, Lee CK, Lee HK, Kennedy BP, Renshaw PF: The Corpus Callosum and Lateral Ventricles in Children wiith Attention-Deficit Hyperactivity Disorder: A Brain Magnetic Resonance Imaging Study. Biol Psychiatry 40: 1060-1063, 1996.

Malone, M.A., Kershner, J.R., Swanson, J.M.: Hemispheric Processing and Methylphenidate Effects in Attention-Deficit Hyperactivity Disorder. J Child Neur 9: 181-189, 1994.

Mefford IN, Potter WZ: A Neuroanatomical and Biochemical Basis for Attention Deficit Disorder with Hyperactivity in Children: A Defect in Tonic Adrenaline Mediated Inhibition of Locus Ceruleus Stimulation. Medical Hypothesis 29: 33-42, 1989.

Morgan AE, Hynd GW, Riccio CA, Hall J: Validity of DSM-IV ADHD Predominantly Inattentive and Combined Types: Relationship to Previous DSM Diagnoses/Subtype Differences. J Am Acad Child Adolesc Psychiatry 35: 325-333. 1996.

Oades RD, Röpcke B, Eggers C: Catecholamine activity reflected in urine of young patients with obsessive compulsive disorder, psychosis with and without reality distortion and healthy subjects: an explorative analysis. J Neural Transmission 96: 143-159, 1994.

Peled O, Carraso R, Globman CH, Yehuda GS: Attention deficit disorder with hyperactivity - changes in hypothalamic function in hyperactive children: a new model. Medical Hypothesis 48: 267-275, 1997.

Pestana M, Soares-Da-Silva P: Outflow of Dopamine and Noradrenaline Originating from L-Dopa and L- threo Dops in Rat Renal Tissues. General Pharmacology 25: 879-885, 1994.

Plitska SR, Maas JW, Javors MA, Rogeness GA, Baker J: Urinary Catecholamines in Attention-Deficit Hyperactivity Disorder with and without Comorbid Anxiety. J Am Acad Child Adolesc Psychiatry 33: 1165-1173, 1994.

Plitska SR, McCracken JT, Maas JW: Catecholamines in Attention-Deficit Hyperactivity Disorder: Current Perspectives. J Am Acad Child Adolesc Psychiatry 35: 264-272, 1996.

Rogeness GA, Maas JW, Javors MA, Macedo CA, Fischer C, Harris WH: Attention Deficit Disorder Symptoms and Urine Catecholamines. Psychiatry Res 27: 241-251, 1989.

Russel V, de Villiers A, Sagvolden T, Lamm M, Talijaard J: Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of Attention-Deficit Hyperactivity Disorder - the spontaneously hypertensive rat. Brain Research 676: 343-351, 1995.

Sallee FR, Stiller RL, Perel JM: Pharmacodynamics of Pemoline in Atttention Deficit Disorder with Hyperactivity. J Am Acad Child Adolesc Psychiatry 31: 244-251, 1992.

Scerbo AS, Kolko DJ: Salivary Testosterone and Cortisol in Disruptive Children: Relationship to Aggressive, Hyperactive, and Internalizing Behaviors. J Am Acad Child Adolesc Psychiatry 33: 1174-1184, 1994.

Schneider SC, Tan G: Attention-deficit hyperactivity disorder (In pursuit of diagnostic accuracy). Postgraduate Medicine 101: 231-240, 1997.

Schulz KP, Halperin JM, Newcorn JH, Sharma V, Gabriel S: Plasma Cortisol and Aggression in Boys With ADHD. J Am Acad Child Adolesc Psychiatry 36: 605-609, 1997.

Semrud-Clikeman M, Biederman J, Sprich-Buckminster S, Lehman BK, Faraone SV, Norman D: Comorbidity between ADDH and Learning Disability: A Review and Report on a Clinically Referred Sample. J Am Acad Child Adolesc Psychiatry 31: 439-448, 1992.

Seri I: Cardiovascular, renal and endocrine actions of dopamine in neonates and children. J Pediatrics 126: 333-344, 1995.

Shaffer D, Fisher P, Dulcan MK, Davies M, Piacentini J, Schwab-Stone ME, Lahey BB, Bourdon K, Jensen PS, Bird HR, Camino G, Reiger DA: The NIMH Diagnositc Interview Schedule for Children Version 2.3 (DISC-2.3): description, acceptability, prevalence rates, and performance in the MECA study. Methods for the Epidemiology of Child and Adolescent Mental Disorders Study. J Am Acad Child Adoles Psychiatry 35: 865-877, 1996.

Shaywitz BA, Yager RD, Klopper JH: Selective brain dopamine depletion in developing rats: an experimental model of minimal brain dysfunction. Science 191: 305-308, 1976.

Sherman DK, Iacono WG, McGue MK: Attention -Deficit Hyperactivity Disorder Dimensions: A Twin Study of Inattention and Impulsivity-Hyperactivity. J Am Acad Child Adolesc Psychiatry 36: 746-753, 1997a.

Sherman DK, McGue MK, Iacono WG: Twin Concordance for Attention Deficit Hyperactivity Disorder: A Comparison of Teachers' and Mothers' Reports. Am J Psychiatry 154: 532-535, 1997b.

Stevenson J: Evidence for a Genetic Etiology in Hyperactivity in Children. Behav Genetics 22: 337-341, 1992.

Stoff DM, Friedman E, Pollock L, Vitiello B, Kendall PC, Bridger WH: Elevated Platelet MAO is Related to Impulsivity in Disruptive Behvaior Disorders. J Am Acad Child Adolesc Psychiatry 28: 754-760, 1989.

Tassin JP: Norepinephrine-Dopamine Interactions in the Prefrontal Cortex and Ventral Tegmental Area: Relevance to Mental Diseases. Advances in Pharmacology 42: 712-716, 1998.

Tassin JP: NE/DA interactions in prefrontal cortex and their possible roles as neuromodualtors in schizophrenia. J. Neural Transmission Suppl. 36: 136-162, 1992.

Tennes K, Kreye M, Avitable N, Wells R: Behavioral Correlates of Excreted Catecholamines and Cortisol in Second-Grade Children. J Am Acad Child Psychiatry 25: 764-770, 1986.

Thierry AM, Pirot S, Gioanni Y, Glowinski J: Dopamine Function in the Prefrontal Cortex. Advances in Pharmacology 42: 717-720, 1998.

Valentino RJ, Foote SL, Page ME: The Locus Coeruleus as a Site for Integrating Corticotropin-Releasing Factor and Noradrenergic Mediation of Stress Responses: Annals New York Acad Sciences 697: 173-188, 1993.

Virkkunen M: Urinary free cortisol secretion in habitually violent offenders. Acta Psychiatr Scand 72: 40-44, 1985.

de Villiers AS, Russel VA, Sagvolden T, Searson A, Jaffer A, Talijaard JJF: α_2-Adrenoreceptor Mediated Inhibition of [^{3}H] Dopamine Release from Nucleus Accumbens Slices and Catecholamine Levels in a Rat Model for Attention-Deficit Hyperactivity Disorder. Neurochemical Research 20: 427-433, 1995.

Wechsler D: Wechscler Intelligence Scale for Children-Revised. New York, NY: Psychological Corp; 1974.

Weinberg WA, Brumback RA: The Myth of Attention Deficit-Hyperactivity Disorder: Symptoms Resulting from Multiple Causes. J Child Neur 7: 431-442, 1992.

Wender PH, Reimherr FW: Buproprion Treatment of Attention-Deficit Hyperactivity Disorder in Adults. Am J Psychiatry 147: 1018-1020, 1990.

Wultz B, Sagvolden T: The Hyperactive Spontaneously Hypertensive Rat Learns to Sit Still, but Not to Stop Bursts of Responses with Short Interresponse Times. Behavior Genetics 22: 415-433, 1992.

Yanagihara N, Izumi F, Miyamato E, Oka M: Factors Affecting Adrenal Medullary Catecholamine Biosynthesis and Release. Advances in Pharmacology 42: 18-21, 1998.

Zametkin AJ, Rapoport JL: Neurobiology of Attention Deficit Disorder with Hyperactivity: Where Have We Come in 50 Years? J Am Acad Child Adolesc Psychiatry 26: 676-686, 1987.

Ziegler MG, Aung M, Kennedy B: Sources of human urinary epinephrine. Kidney International 51: 324-327, 1997.

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